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SAS6-like protein in *Plasmodium* indicates that conoid-associated apical complex proteins persist in invasive stages within the mosquito vector

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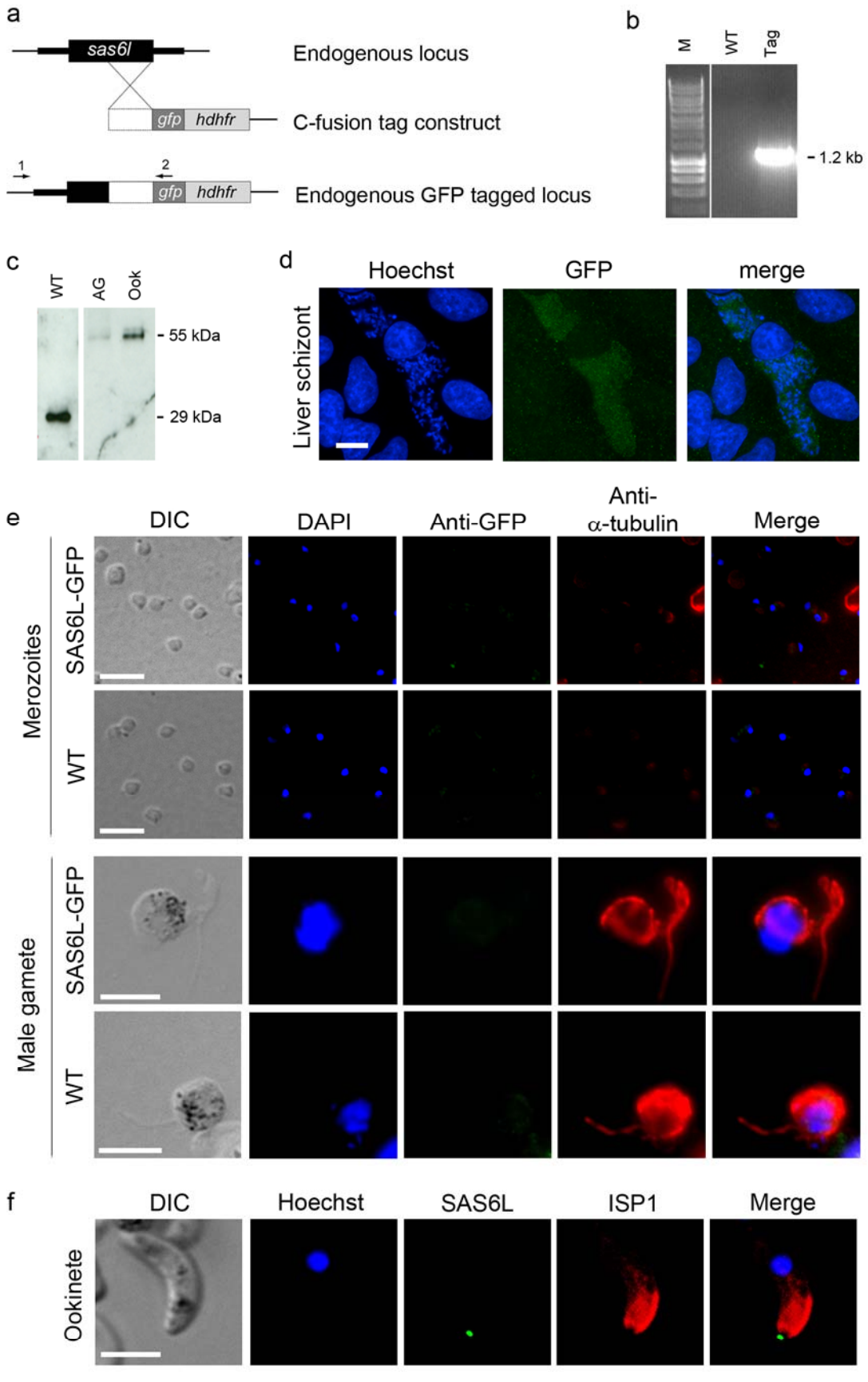
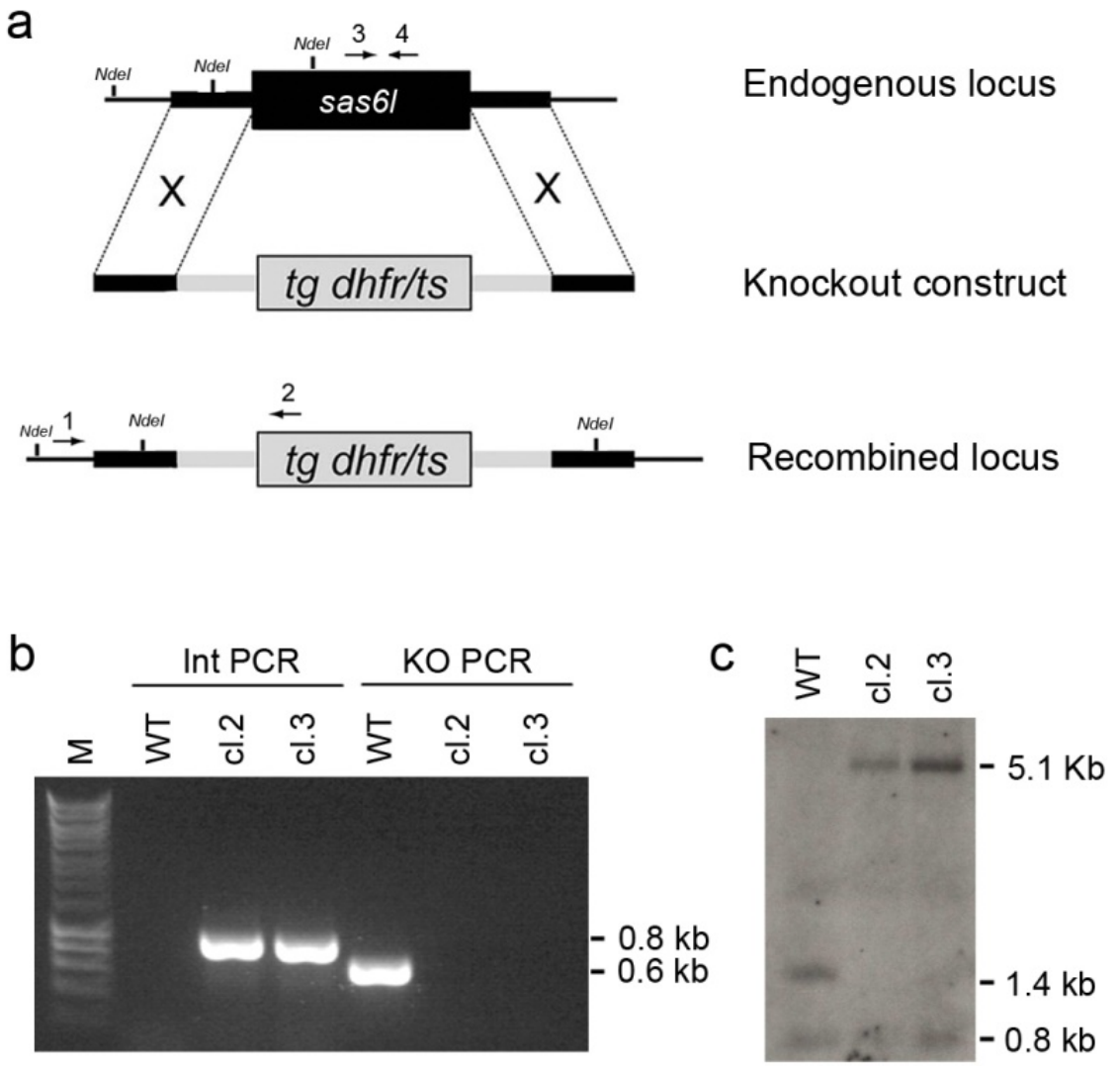


g

>HA-APEX
MGYPYDVPDYAGKSYPTVSADYQDAVEKAKKKLRGFIAEKRCAPLMLRLAFH
SAGTFDKGTKTGGPFGTIKHPAELAHSANNGLDIAVRLLEPLKAEFPILSYADF
YQLAGVVAVEVTGGPKVPFHPGREDKPEPPPEGRLPDATKGSDHLRDVFGK
AMGLTDQDIVALSGGHTIGAAHKERSGFEGPWTSNPLIFDNSYFTELLSGEKE
GLLQLPSDKALLSDPVFRPLVDKYAADEDAFFADYAEAHQKLSELGFADA

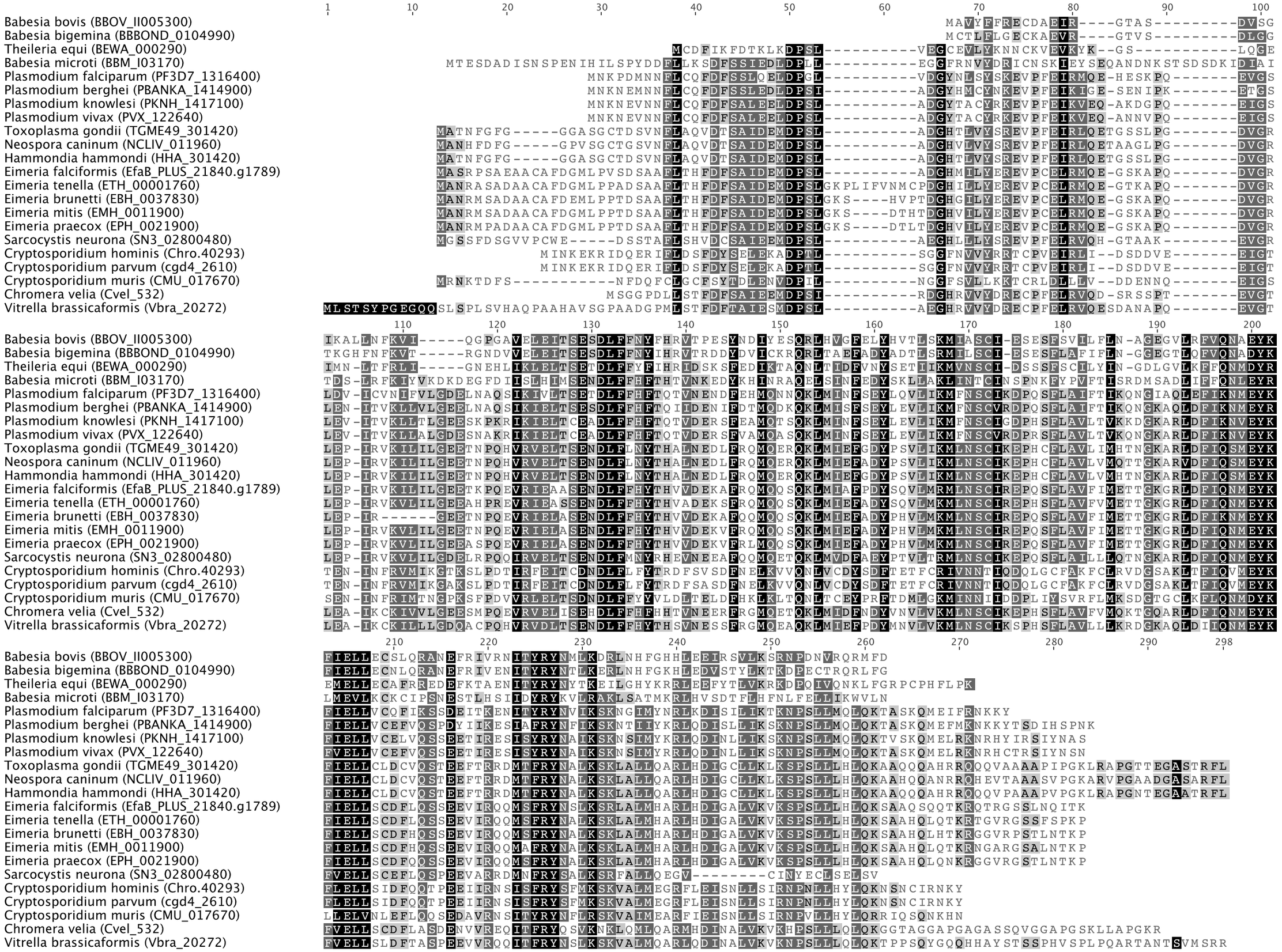
Supplementary Fig. S1: Generation, genotypic analysis and immunofluorescence images of SAS6L-GFP parasite line

(**a**) Schematic representation of the endogenous *sas6l* locus, the GFP-tagging construct and the recombined *sas6l* locus following single homologous recombination. Arrows 1 and 2 indicate the PCR primers used to confirm successful integration of the construct. (**b**) Diagnostic PCR of SAS6L-GFP and WT parasites using primers IntT174 (Arrow 1) and ol492 (Arrow 2). Integration of *the sas6l* tagging construct gives a band of 1.2 kb. TAG = SAS6L-GFP parasite line. (**c**) Western blot after GFP-Trap of SAS6L-GFP (55 kDa) and WT-GFP (29 kDa) protein to illustrate SAS6L-GFP in activated gametocytes (AG) and ookinetes (Ook) parasite stages. (**d**) Live fluorescent imaging of 54 hrs liver stage mature schizont with merozoites using the SAS6L-GPF parasite line. Nuclei were detected using Hoechst 33342 (blue). Merge is the composite of Hoechst and GFP signals. Scale bar = 10 µm. (**e**) Immunofluorescence images of blood stage merozoites and a male gamete for SAS6L-GFP and WT (not expressing GFP in any form) parasite lines. Green channel represents the anti-GFP antibody staining, red channel represents the anti-α-tubulin antibody staining. Nuclei were detected using DAPI in the vectashield mounting media (blue), and the cells were displayed by differential interference contrast (DIC). Merge is the composite of DAPI, anti-GFP and anti-α-tubulin antibodies. (**f**) Live imaging of dual tagged ookinetes using SAS6L-GFP (green channel) and ISP1-mCherry (red channel) parasite lines. Nuclei were detected using Hoechst 33342 (blue), and the cells were displayed by DIC. Merge is the composite of Hoechst, GFP and mCherry. Scale bars = 5 µm. (**g**) Protein sequence the C-terminal fusion HA-APEX (hemagglutinin epitope fused to an engineered ascorbate peroxidase) tag appended to *T. gondii* SAS6L.



Supplementary Fig. S2: Generation and genotypic analysis of Δ*sas6l* mutant parasite line

(**a**) Schematic representation of the endogenous *sas6l* locus, the targeting gene deletion construct and the recombined *sas6l* locus following double homologous recombination. Arrows 1 and 2 indicate PCR primers used to confirm successful integration of the construct and arrows 3 and 4 indicate PCR primers used to confirm deletion of the *sas6l* gene in both clones. (**b**) Diagnostic PCR of the *sas6l* locus in Δ*sas6l* and WT parasites using primers IntN90 (Arrow 1) and ol248 (Arrow 2). Integration of the targeting construct gives a band of 0.8 kb. Presence of the gene gives a band of 0.6 kb (Arrows 3 and 4; N90KO1 and 2). (**c**) Southern blot analysis of Δ*sas6l* and WT parasite genomic DNA following *Nde*I digestion. A probe specific for the *sas6l* 5'UTR bound to a 1.4 kb band in WT, to a 5.1 kb band in Δ*sas6l* parasites and to a 0.8 kb band in all parasite lines. Data for Δ*sas6l* clone 2 and 3 (cl.2/3) are shown.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Babesia bigemina (BBBOND_0104990)
Theileria equi (BEWA_000290)
Babesia microti (BBM_I03170)
Plasmodium falciparum (PF3D7_1316400)
Plasmodium berghei (PBANKA_1414900)
Plasmodium knowlesi (PKNH_1417100)
Plasmodium vivax (PVX_122640)
Toxoplasma gondii (TGME49_301420)
Neospora caninum (NCLIV_011960)
Hammondia hammondi (HHA_301420)
Eimeria falciformis (EfaB_PLUS_21840.g1789)
Eimeria tenella (ETH_00001760)
Eimeria brunetti (EBH_0037830)
Eimeria mitis (EMH_0011900)
Eimeria praecox (EPH_0021900)
Sarcocystis neurona (SN3_02800480)
Cryptosporidium hominis (Chro.40293)
Cryptosporidium parvum (cgd4_2610)
Cryptosporidium muris (CMU_017670)
Chromera velia (Cvel_532)
Vitrella brassicaformis (Vbra_20272)
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Supplementary Figure S3: Alignment of select SAS6L proteins from apicomplexans and chromerids.

Supplementary Table S1: Oligonucleotides used in this study

Name	Sequence 5'-->3'	Notes
T1741	CCCCGGTACCGAGCTAACTAGCGAATCCGATTTATTC	KpnI site underlined
T1742	CCCCGGGCCCCTTATTTGGGCTGTGTATATCACTTG	ApaI site underlined
IntT174	GGTGAGGAATTAAATGCACAAAG	
ol492	ACGCTGAACTTGTGGCCG	
N0901	CCCCGGGCCCGGAAGATTTAGATCCATCAATAG	ApaI site underlined
N0902	GGGGAAGCTTGTCATTTTTGATCTAAGATG	HindIII site underlined
N0903	CCCCGAATTCGCAACTTCAAAAAACAGC	EcoRI site underlined
N0904	GGGGTCTAGAGGCAAAACAAACTCTTCGCAAC	XbaI site underlined
IntN90	GGTAATAATAAGGTTGAATAAAATTG	
ol248	GATGTGTTATGTGATTAATTCATACAC	
N90 ko1	GTGTATATATTTATTTCCATTTTTTAG	
N90 ko2	CCGGGGATTGAACAAATTCACACAC	
hsp70 FW	GTATTATTAATGAACCCACCGCT	PBANKA_081890
hsp70 RV	GAAACATCAAATGTACCACCTCC	
arginyl-tRNA FW	TTGATTCATGTTGGATTTGGCT	PBANKA_143420
arginyl-tRNA RV	ATCCTTCTTTGCCCTTTCAG	
seryl-tRNA FW	CAACCGCTATGCGCATTACAC	PBANKA_061540
seryl-tRNA RV	CTCAACCTTATCAAACTGATGAACT	
nek2 FW	AGAGGCATTTATTGAAGACGG	PBANKA_124070
nek2 RV	GCTGTAATTATCTGTAGCAACCA	
nek4 FW	GATGTATGGGCTATTGGCT	PBANKA_061670
nek4 RV	AGCATAACTGTTGAATTCCCT	
isp1 FW	GCCACCAAAAGGTACGAATG	PBANKA_120940
isp1 RV	GCCAAACAACAATTGCCACT	
isp3 FW	AGCTTGTGCTGCATTAACGA	PBANKA_132430
isp3 RV	TTGAATTTCATTTCCATCAGGA	
ppkl FW	TTCTAAAGTACCTTCACCAAGAG	PBANKA_132950
ppkl RV	TAGCAGGTCCTTCTTTACAC	
dozi FW	GCAAGAATGTCGCAAACAC	PBANKA_121770
dozi RV	TCTGAGGAAACTAAACATCGAC	
sas6 FW	GAACAATATCACTGCATCCCC	PBANKA_010620
sas6 RV	GCTGGTGTGTAACCTATTCCT	
sas6l FW	GTAAAATTATTAGTTCTAGGTGAGGAATT	PBANKA_141490
sas6l RV	GTTTGTCTTGCATTGTATCGAATATG	
Tgsas6l FW	TGGTCTCAGGAGCTTCTGCTCTTGTTCTGCGTGTATTTC	TGME_49301420
Tgsas6l RV	TGGTCTCACGAAAGGAACCGAGTGGATGC	